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Effects of various AMF and *Bacillus pumilus* strain NBRC 12092 on *Ocimum basilicum* grown under salinity stress

Nidhi Yadav

Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India

Abstract
The aim of the present study was to assess the synergistic effects of Arbuscular Mycorrhizal fungi (AMF) and *Bacillus pumilus*, on growth parameters of test plant *Ocimum basilicum* grown under 40 ppm of Sodium fluoride stress. Plant growth promotory rhizobacteria when grown in association with various mycorrhizal fungi showed a remarkable increase in plant height, leaf fresh weight, leaf dry weight and total fresh biomass. *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 increased leaf fresh weight by 21.5% and 18% respectively as compared to control. Whereas, consortium of *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 (Gm+St 2) resulted in 24% increase in leaf fresh weight of *Ocimum basilicum* as compared to control. Best consortium results were shown by *G. mossae* followed by *G. fasciculatum, G. intraradices* and *G. aggregatum.* Inoculation of tulsi seedlings with mycorrhizal fungi and *Bacillus pumilus* increased the fluoride tolerance level of the herb.

Keywords: arbuscular mycorrhizal fungi (AMF), *Bacillus pumilus*, Consortium, Sodium fluoride, *Ocimum basilicum*

Introduction
Medicinal herbs have been used from pre-historic times (Dragland *et al.* 2003) [3]. From ancient time, Tulsi is used as a traditional remedy for wound healing and microbial infections. *Ocimum basilicum* is a medicinal and aromatic plant (MAP), belonging to family *Lamiaceae* (Grayer *et al.* 1996) [6]. It is an annual herb native to India and other parts of Asia (Klimankova *et al.* 2008) [9]. The use of plant parts like root, stem and leaves has been maintained traditionally (Leonti *et al.* 2003). Mycorrhizal fungi are found everywhere. These symbionts attach and become part of the plant. AM fungi enhance the nutrient uptake and crop yield by solubilizing phosphate. If there is high concentration of phosphorous in the soil, mycorrhiza never die rather they control the phosphorus content of the soil (Smith *et al.* 1997) [14].

Bacillus pumilus strain NBRC 12092 is a gram positive, aerobic, rod shaped and endospore forming bacteria (Ghosh *et al.* 2007) [5]. It shows high resistance to environmental stress. Fluorine is the most electronegative atom, and therefore has the ability to make strong hydrogen bonds. Fluoride accumulation, in even low concentrations can cause an abnormal change in biochemical and physiological parameters in plants and animals. In higher concentrations, it causes dental and skeletal fluorosis in humans (Lakshmi 2013) [10]. Plant tolerance can be enhanced by AMF and their mutual relationship with plants help in their growth even in stressed conditions through improved physiological and nutritional stress (Hashem *et al.* 2015, 2016) [7, 8]. In this study, we studied the impact of fluoride stress on *Ocimum basilicum.* And also the ameliorating effects of various species of *Glomus* and *Bacillus pumilus* strain NBRC 12092 on *Ocimum basilicum.*

Taxonomic Classification
Basil Tulsi ***(Ocimum basilicum)***

Kingdom	:	Plantae
Phylum	:	Angiosperms
(unranked)	:	Eudicots
Class	:	Asterids
Order	:	Lamiales
Family	:	Lamiaceae/Labiateae
Genus	:	*Ocimum*
Species	:	*basilicum*

Material and Method
Sample collection
A bacterium, *Bacillus pumilus* strain NBRC 12092, was isolated from the fluoride affected soil of Sirsahakhera region of Unnao district in Uttar Pradesh, India and was tested against different Sodium fluoride (NaF) concentrations: 100ppm, 200ppm, 300ppm, 400ppm and 500ppm.

Experimental site
The experimental site, Lucknow (Uttar Pradesh), with a warm humid subtropical climate is situated in the north-eastern part of Uttar Pradesh, India. Latitude: 26°50′21″ N, Longitude: 80°55′23″ E and Elevation above sea level: 126 m = 413 ft. The average annual rainfall of this area is 313 mm, which is evenly distributed from June to October and August is the wettest month of the year. The average temperature ranges from 26°C to 39°C and actual temperature ranges from 29°C to 47°C. The relative humidity fluctuated between 34 % and 92%.

Seed treatment
Seeds of *Ocimum basilicum* were obtained from the National Gene Bank for Medicinal and Aromatic Plants at the CSIR-

Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India. *Ocimum basilicum* seeds were surface sterilized with 10% NaOCl for 5 minutes and were properly rinsed with distilled water for 5 times before sowing.

Experimental set-up

Seeds of *O. basilicum* were sown in the polyethylene bags filled with sterile soil. 15 days old seedlings were then transferred to the earthen pots containing 1kg soil by using *5* g of inoculum of different AM fungi per seedling respectively placed at 5cm depth in pots. The seedlings were dipped in the bacterial inoculum solution of *Bacillus pumilus* for 30 min and were transferred to the pots having AM Fungi. The experimental set up was in completely randomized block design with three replicates of each, i.e. control and treatments in a glass house. The inoculum of different *Glomus* fungi (*Glomus mossae, Glomus fasciculatum, Glomus intraradices and Glomus aggregatum)* were obtained from CSIR-Central institute of medicinal and aromatic plants, Lucknow (U.P)

Determination of Plant physical parameters

After 2 months, the crop was harvested to determine various physical parameters.

Statistical analysis

The collected data was subjected to statistical analysis for analysis of variance method (ANOVA), suitable to completely randomized design (CRD) for pot experiment with the help of software ASSISTAT 7.7 beta version. Microsoft excel was used for calculating Standard deviation and Standard error. The means were calculated using Duncan's multiple range tests under a significance level of $P \leq 0.05$.

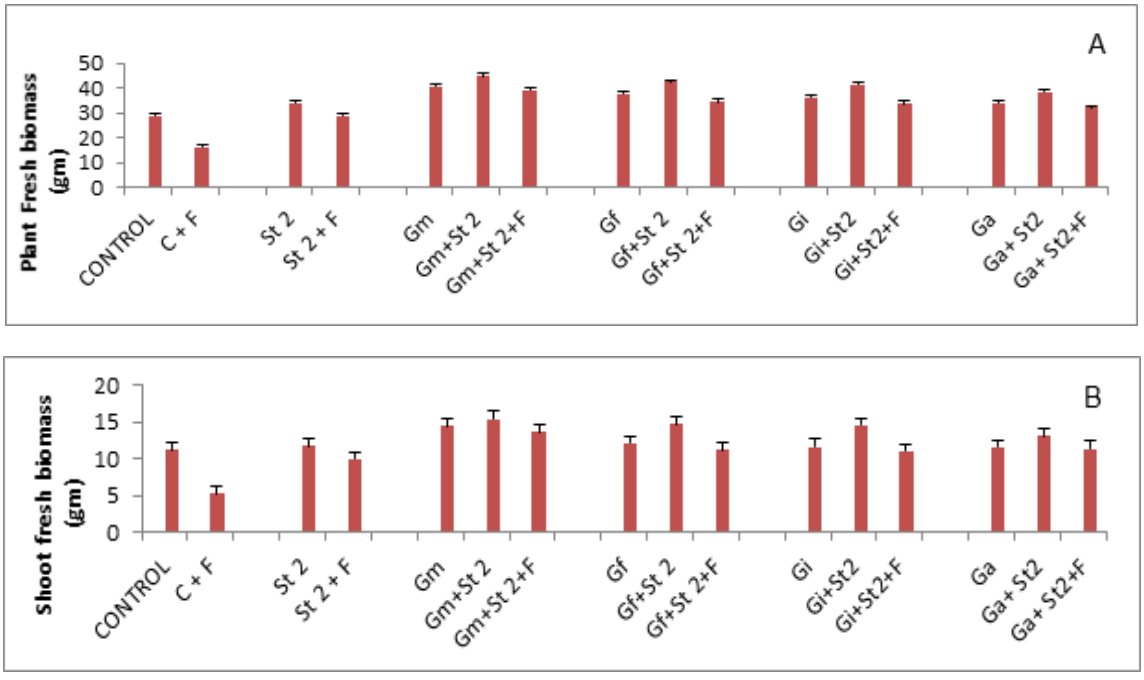
Result

Table 1: Effects of various treatments involving different AMF and *Bacillus pumilus* strain NBRC 12092 on *Ocimum basilicum* grown under NaF stress.

S. No	Treatments	Plant fresh biomass (gm)	Shoot fresh biomass (gm)	Leaf fresh biomass (gm)	Leaf dry weight(gm)	% Oil yield
1	CONTROL	28.78h	11.253e	16.106g	2.806e	0.530j
2	C + F	16.34i	5.256g	9.143h	1.120g	0.461m
3	St 2	33.69fg	11.717e	19.124e	3.018de	0.583h
4	St 2 + F	28.75h	10.015f	16.258g	2.344f	0.489l
5	Gm	40.56bc	14.410ab	22.533bc	4.146bc	0.682d
6	Gf	37.52de	12.110de	22.186cd	3.990bc	0.644ef
7	Gi	36.22e	11.650e	21.486d	3.806c	0.611g
8	Ga	34.03f	11.580e	19.363e	3.096de	0.567i
9	Gm+St 2	44.70a	15.483a	25.243a	4.823a	0.788a
10	Gm+St 2+F	39.11cd	13.630bc	21.886d	3.940c	0.712c
11	Gf+St 2	42.35b	14.790a	23.733b	4.346b	0.734b
12	Gf+St 2+F	34.40f	11.226e	19.710e	3.290d	0.647e
13	Gi+St 2	41.46b	14.563ab	23.233bc	4.170bc	0.689d
14	Gi+ St 2+F	33.64fg	11.080e	19.250e	3.193de	0.587h
15	Ga+St 2	38.33d	13.070cd	21.786d	3.913c	0.635f
16	Ga+St 2+F	31.94g	11.426 e	17.593f	2.926de	0.527j

*Values denoted by same letter are not significantly different at $P<0.05$ level

Where F: Sodium fluoride, St 2: *Bacillus pumilus* strain NBRC 12092, Gm: *Glomus mosseae*, Ga: *Glomus aggregatum*, Gf: *Glomus fasciculatum*, Gi: *Glomus intraradices*.



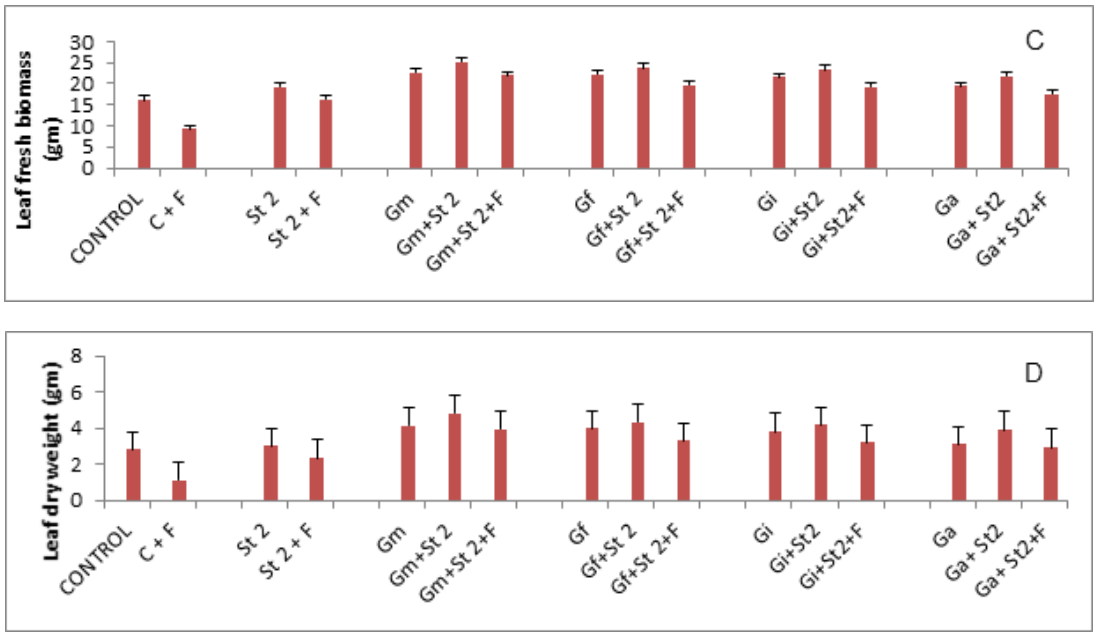


Fig 1: Histograms showing effect of various AM fungi and *Bacillus pumilus* strain NBRC 12092 on A.) Plant fresh biomass, B.) Shoot fresh biomass, C.) Leaf fresh biomass, D.) Leaf dry weight of *Ocimum basilicum.* Where F: Sodium fluoride, St 2: *Bacillus pumilus* strain NBRC 12092, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gi: *Glomus intraradices*, Ga: *Glomus aggregatum*.

Discussion

Sodium fluoride has an inhibitory effect on plant growth. Total plant fresh biomass decreased to 28% in fluoride affected plants as compared to control. Neutral to alkaline pH favors germination of *G. mossae.* Plants inoculated with AM fungi growing under saline conditions resulted in an increase in root length, fresh and dry weights of shoot and increased photosynthesis (Shhekoofeh and Sepideh 2011). *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 increased leaf fresh weight by 22.5% and 18% respectively. Consortium of *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 (Gm+St 2) resulted in 24.2% increase in leaf fresh weight of *Ocimum basilicum* as compared to control. Synergistic effects of AM fungi and *Bacillus pumilus* showed a remarkable coping effect of the herb against fluoride stress. Even in the presence of fluoride stress, the consortium showed a remarkable increase of 21% in leaf fresh weight. The best coping effect was shown by *G.mossae* followed by *G.fasiculatum*, *G. intraradices* and *G.aggregatum* respectively. Studies show that AM fungi are the root symbionts which improve plant health and mineral nutrition (Smith and Read 1997) [14]. So the results were coherent with the findings of earlier studies.

Conclusion

Being a medicinally important plant, leaves of *Ocimum basilicum* are rich in essential oil. Synergistic effect of AM fungi (AMF) inoculation with *Bacillus pumilus* under fluoride stress resulted in increase in total fresh biomass, leaf & shoot fresh weights and leaf dry weight by coping up with stress effects of fluoride. AM fungi and *Bacillus pumilus* used synergistically may prove to be a panacea for better yielding of natural herbs in stress conditions. Ecosystem services provided by these microbial consortia may not only reduce the use of hazardous chemicals in agriculture but would also improve microbial diversity resulting in crop productivity and an eco-friendly approach of sustainable organic farming.

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